SUPPLEMENTARY INFORMATION for

Specific factors in blood from young but not old mice directly promote synapse formation and NMDA-receptor recruitment

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This PDF file includes:

Supplementary Information Materials and Methods
Figs. S1 to S5
Supplementary Information References

Supplementary Information Materials and Methods

Table of reagents, plasmids, and software

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-Synapsin (YZ6078)	Yenzym Antibodies	N/A
Rabbit polyclonal anti-Synapsin	T.C. Südhof, Stanford University	Cat# E028; RRID: AB_2315400
Chicken polyclonal anti-MAP2A/B	EnCor Biotechnology	Cat# CPCA-MAP2; RRID:AB_2138173
Rabbit polyclonal anti-NeuN	Millipore	Cat# ABN78; RRID:AB_10807945
Sheep polyclonal anti-Thrombospondin-4	R&D Systems	Cat# AF7860; RRID: AB_2202085
Goat polyclonal anti-SPARC-like protein-1	R&D Systems	Cat# AF2836; RRID: AB_2195097
IRDye 680RD Goat anti-mouse Immunoglobulin (heavy + light chains)	Li-Cor	Cat# 925-68070; RRID: AB_10956589
Biological Samples		
HyClone fetal bovine serum (U.S.), characterized	Fisher Scientific	Cat# SH3007103
Chemicals, Peptides, and Recombinant Proteins		
Tetrodotoxin	Tocris	Cat# 1078; CAS: 4368-28-9
Picrotoxin	Tocris	Cat# 1128; CAS: 124-87-8
CNQX	Tocris	Cat# 1045; CAS: 479347-85-8
D-AP5	Tocris	Cat# 0106; CAS 79055-68-8
Critical Commercial Assays		
ProteoExtract Albumin/IgG Removal Kit	Calbiochem	Cat# 122643
TMTsixplex Isobaric Label Reagent Set	Thermo Fisher Scientific	Cat# 90061
In-Fusion HD Cloning Kit	Takara Bio	Cat# 121416
Experimental Models: Cell Lines		
H1 human embryonic stem cells	WiCell	Cat# WA01
Experimental Models: Organisms/Strains		
Mouse: CD1 wildtype	Charles River	Cat# Crl:CD1(ICR)
Recombinant DNA		
L201-Syn-GFP-Homer1	Dr. Rick Sando, Südhof Lab, Stanford University	N/A
pmU-B-actin-eBFP	Dr. Gary Banker, Oregon Health and Sciences University	N/A
pEBMulti-Neo	Wako	Cat# 057-08131
pCMV-SPORT6-Alpha-2-HS glycoprotein	Dharmacon	Cat# MMM1013-202765025; MGC 13882

pCMV-SPORT6-Lumican	Dharmacon	Cat# MMM1013-202762407; MGC 5828
pCMV6-Entry-Thrombospondin 4	OriGene	Cat# MR219213
pCMV-SPORT6-Alpha-fetoprotein	Dharmacon	Cat# MMM1013-202858350; MGC 76732
pCMV-SPORT6-SPARC-like protein 1	Dharmacon	Cat# MMM1013-202761476; MGC 5894
pCR-BluntII-TOPO-Comp protein	Dharmacon	Cat# MMM1013-211694158; MGC 150282
pCMV-SPORT6-Pregnancy zone protein	Dharmacon	Cat# MMM1013-202768721; MGC 65284
pYX-Asc-Complement factor H	Dharmacon	Cat# MMM1013-202859750; MGC 90130
pCMV-SPORT6-Alpha-1B glycoprotein	Dharmacon	Cat# MMM1013-202701712
pCMV6-Entry-Complement factor I	Origene	Cat# MR216342
Software and Algorithms		
NIS-Elements Confocal	Nikon	https://www.nikoninstruments.com/Products/Software/NIS-Elements-Confocal
Metamorph 7.7.0	Molecular Devices	https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metamorph-microscopy#gref
Image Studio 5.2.5	Li-Cor	https://www.licor.com/bio/products/software/image_studio
Prism 8	Graph Pad	https://www.graphpad.com/scientific-software/prism/
Microsoft Excel	Microsoft	https://products.office.com/en-us/excel

Animal procedures and serum collections. Male and female CD1 wild-type mice Crl:CD1 (ICR), obtained from Charles River Laboratories, were used for dissociated glia cultures and serum collections. For serum collections, young (postnatal day 15; P15) and aged (12-15 months old) CD1 mice were euthanized by CO_2 inhalation, and blood was withdrawn by cardiac puncture and exsanguination. After clotting for 30 min at room temperature, blood samples were centrifuged at 13,000 x g for 4 min, and the aqueous upper phase was collected. Centrifugation was repeated to remove residual erythrocytes, and the clarified serum was stored at -80^{o}C. All animal procedures were approved by the Administrative Panel on Laboratory Animal Care (APLAC) at Stanford University and are consistent with the policies of the Public Health Service

Policy on Humane Care and Use of Laboratory Animals, and the Panel on Euthanasia of the American Veterinary Medical Association. All mice were housed in the Research Animal Facility at the Stanford Institutes of Medicine, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Generation of human neurons from ES cells. Human neurons (iN cells) were generated from H1 ES cells as described (*1-4*) with the following modifications. One day before induction, ES cells were detached with Accutase (Innovative Cell Technologies) and plated on 1.5 mm glass coverslips coated with Matrigel (BD) in 24-well plates. ES cells were plated at high density (2.5 x 10^4 cells/well) for electrophysiology experiments and at low density (1.0 x 10^4 cells/well) for morphological analyses and immunocytochemistry. Concurrently, lentiviruses expressing Ngn2 and rtTA were added to the ES cells (0.5 µL/well). The following day (0 days *in vitro*; DIV), Ngn2 expression was induced with doxycycline (Sigma) in DMEM-F12 medium containing N2 and non-essential amino acids (NEAA; Invitrogen), supplemented with human BDNF (PeproTech), human NT3 (PeproTech), and mouse Laminin-1 (Invitrogen). Doxycycline was retained in the media until neurons were used for experiments. At DIV1, puromycin selection was carried out for 48 h. At DIV3, mouse glia were added to the differentiating neurons in Neurobasal A medium containing B27 and Glutamax (Invitrogen) and Ara-C (Sigma) to inhibit glia proliferation. Mouse glia were cultured from cortices of newborn CD1 mice. Cortices were dissected, digested with papain for 20 min at 37^{o}C, and harshly triturated. Dissociated cells were plated in T75 flasks in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Upon reaching confluence, glial cells were trypsinized and re-plated at a lower density. Re-plating was repeated twice to remove residual mouse neurons before glia were co-cultured with human neurons. Fetal bovine serum (FBS; Hyclone, GE Healthcare) or mouse sera (2.5%; collection procedure described below) were added to the culture medium at DIV10. Neurons were assayed after at least DIV35.

Lentivirus preparation. Lentiviruses for trans-differentiation of ES cells into neurons were generated from the following plasmids: TetO-Ngn2-P2A-puromycin and Ubiquitin-rtTA (Figure S1A; *1*). Lentiviruses were produced in HEK293T cells as described previously (*1*). Briefly, HEK293T cells were transfected using calcium phosphate with each lentiviral plasmid and the three helper plasmids pRSV-REV, pMDLg/pRRE, and VSV-G protein expression vector. The culture medium was harvested 48 h after transfection, and virus particles were pelleted by ultracentrifugation. Pellets were resuspended in DMEM, aliquoted, and stored at -80^{o}C.

Serum protein isolation. Proteins in serum samples from young and aged mice were isolated by centrifugal ultrafiltration through an Amicon regenerated cellulose membrane with a nominal molecular weight limit of 3 kDa (Millipore). Samples were centrifuged at 14,000 x g for 30 min at room temperature, and the protein concentrates were subsequently recovered from the membranes with a reverse spin at 1,000 x g for 2 min at room temperature. Total protein concentrations were measured using a colorimetric BCA protein assay according to the manufacturer's protocol (ThermoFisher Scientific).

Depletion of albumin and immunoglobulin. Serum samples were depleted of albumin and immunoglobulin (IgG) using ProteoExtract columns according to the manufacturer's protocol (Calbiochem). Briefly, 500 µL serum samples were passed by gravity flow through ProteoExtract columns packed with an albumin-specific affinity resin and an immobilized Protein A polymeric

resin. Following two sequential elutions in binding buffer, depleted samples were concentrated by centrifugal ultrafiltration as described above and stored at -80^{o}C. Albumin and immunoglobulin depletion was confirmed by SDS-PAGE gel electrophoresis on a TGX Stain-Free precast gel (Biorad) as an alternative to Coomassie blue staining. Proteins were visualized by UV illumination, which induced covalent binding between trihalo compounds in the gel and tryptophan residues in the proteins to produce fluorescence.

TMT isobaric mass tagging and tandem mass spectrometry. Serum peptides were prepared and labeled with TMT isobaric mass tags according to the manufacturer's protocol (ThermoFisher Scientific). Briefly, depleted serum samples were denatured with 10% SDS, reduced with Tris (2-carboxyethyl) phosphine, and alkylated with iodoacetamide. Proteins were then precipitated with acetone and digested with trypsin. Digested peptides from each serum sample (100 µg per reaction) were differentially labeled with TMT Label Reagents and subsequently combined in equal amounts. Quantitation of labeled peptides was performed using a tandem mass spectrometer capable of MS/MS fragmentation and spectra were analyzed by the Stanford University Mass Spectrometry Facility (SUMS). Similar proteins were consolidated into groups, and ratiometric abundances were determined in reference to the pooled control. To select candidates for further investigation, protein groups were ranked according to coverage, and reciprocal fold-elevation ratios were calculated from their abundances in the serum samples to determine if they were enriched in young or old blood. All candidates exhibited fold-elevation ratios of >1.5.

Cloning and recombinant expression of candidate serum factors. Mouse full-length cDNA clones corresponding to the 10 candidate blood factors selected for screening experiments were obtained from the NIH Mammalian Gene Collection (Dharmacon, GE Healthcare). Inserts comprising each cDNA sequence flanked by 30 bp vector homology arms were generated by PCR, and complementary ends were annealed with linearized pEB-Multi-Neo (Wako) using In-Fusion cloning (Clontech). Expression constructs were transfected into HEK 293T cells using calcium phosphate and incubated in serum-free medium. After 72 h, the culture medium was collected, passed through a 0.2 µm syringe filter to remove cell debris, and concentrated by centrifugal ultrafiltration as described above for serum proteins. Concentrated supernatants were diluted 1:100 in serum-free neuronal growth medium and added to human neuron cultures at 35 DIV. Neurons were assayed within 5-7 days after treatment.

Immunoblotting of recombinant proteins and serum proteins. Supernatants from transfected HEK 293T cells were collected and precipitated with trichloroacetic acid and acetone. Protein pellets were resuspended in sample buffer (200 mM Tris-Cl pH 6.8, 8% SDS, 0.4% bromophenol blue and 40% glycerol) and boiled for 10 min. Recombinant proteins were analyzed by SDS-PAGE in the presence of β-mercaptoethanol. Immunoblotting and quantitative analysis were performed by a dual-channel infrared imaging system with fluorescence-labeled secondary antibodies (800CW and 680LT), an Odyssey Infrared Imager CLX and software Image Studio 5.2.5 (LI-COR Biosciences). Serum proteins were diluted 1:5 prior to mixing with sample buffer and were henceforth analyzed similarly. Serum protein signals were normalized to immunoglobulin heavy chain, probed on the same blots as loading controls. Antibodies used are listed in the Reagent Table.

Immunocytochemistry. All immunocytochemistry experiments were performed as described (*5*). Briefly, human neurons cultured at low density were fixed with 4% paraformaldehyde for 15 min at 37ºC, permeabilized with 0.1% Triton-X for 10 min at room temperature, and blocked with 0.5% fish skin gelatin for 1 h at 37ºC. To measure dendritic length and branching and to quantify synapses, neurons were co-stained with anti-MAP2 (1:1000; EnCor Biotechnology) and anti-synapsin (1:500; YenZym). Neuronal nuclei were identified by staining with anti-NeuN (1:500; Millipore). All primary antibodies were diluted in blocking solution and applied overnight at 4ºC with gentle agitation. Neurons were subsequently incubated with compatible secondary antibodies conjugated to Alexa-488 and Alexa-546 fluorophores (1:500, Invitrogen) for 1.5 h at 37ºC.

Image acquisition and analysis. Images of neurons with pyramidal morphology were acquired using a Nikon A1RSi confocal microscope with constant laser gain and offset settings, scanning speed, and pinhole size. These settings yielded images in which the brightest pixels were not oversaturated. Each Z-stack was comprised of 10 serial images acquired in 0.5-µm steps. Maximum intensity projections of the Z-stacks were generated for quantification purposes. Synaptic puncta were counted along well isolated primary dendrites (5 x 100-µm dendritic segments per cell) using the "Count Nuclei" application in Metamorph (Molecular Devices). To measure dendritic length and branching, field images of low-density neuronal cultures stained with MAP2 and NeuN were analyzed using the "Neurite Outgrowth" application in Metamorph. Constant threshold settings to exclude background signals were maintained for all experimental conditions.

Electrophysiology. Electrophysiological recordings were performed in the whole cell configuration as described previously (*1-4*, *6*). Patch pipettes were pulled from borosilicate glass capillary tubes (Warner Instruments) using a PC-10 pipette puller (Narishige). The resistance of pipettes filled with intracellular solution varied between 3.0-4.0 MΩ. The bath solution in all experiments contained (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH adjusted to 7.4 with NaOH, 300 mOsm/L). Intrinsic firing properties were recorded in current-clamp mode. Minimal currents were introduced to hold membrane potentials at -70 mV. To induce action potentials, increasing currents were injected for 1 s in an incremental manner. The pipette solution used for these recordings contained (in mM): 123 K-gluconate, 10 KCl, 1 $MgCl_2$, 10-HEPES, 1 EGTA, 0.1 $CaCl_2$, 1.5 MgATP, 0.2 Na_4GTP and 4 glucose (pH adjusted to 7.2 with KOH; 310 mOsm/L). Spontaneous miniature and evoked synaptic responses were recorded in voltage-clamp mode with a pipette solution containing (in mM): 135 CsC1, 10 HEPES, 5 EGTA, 4 MgATP, 0.3 Na_4GTP and 4 glucose (pH adjusted to 7.2 with CsOH; 310 mOsm/L). Evoked synaptic responses were triggered by 0.5-ms current (100 µA) injection through a local extracellular electrode (FHC concentric bipolar electrode) placed 100-150 µm from the soma of neurons recorded. The frequency, duration and magnitude of the extracellular stimulus were controlled with a Model 2100 Isolated Pulse Stimulator (A-M Systems, Inc.) synchronized with the Clampex 10.1 data acquisition software (Molecular Devices). Spontaneous miniature excitatory postsynaptic currents (mEPSCs) were monitored in the presence of tetrodotoxin (1 µM). Evoked EPSCs were pharmacologically isolated with picrotoxin (50 uM) and recorded at -70 mV or +40 mV holding potentials. Data were digitized at 10 kHz with a 2 kHz low-pass filter using a

Multiclamp 700B Amplifier (Molecular Devices). Data were analyzed using Clampfit 10.1 software. All experiments were performed at room temperature.

Statistical analyses. All experiments were performed on at least 15 cells from 3 independent cultures. All data shown are means ±SEMs. All statistical analyses were performed using one- or two-way ANOVAs with Tukey's post-hoc tests, comparing control and treated conditions within the same experiments.

Supplementary Figures and Figure Legends

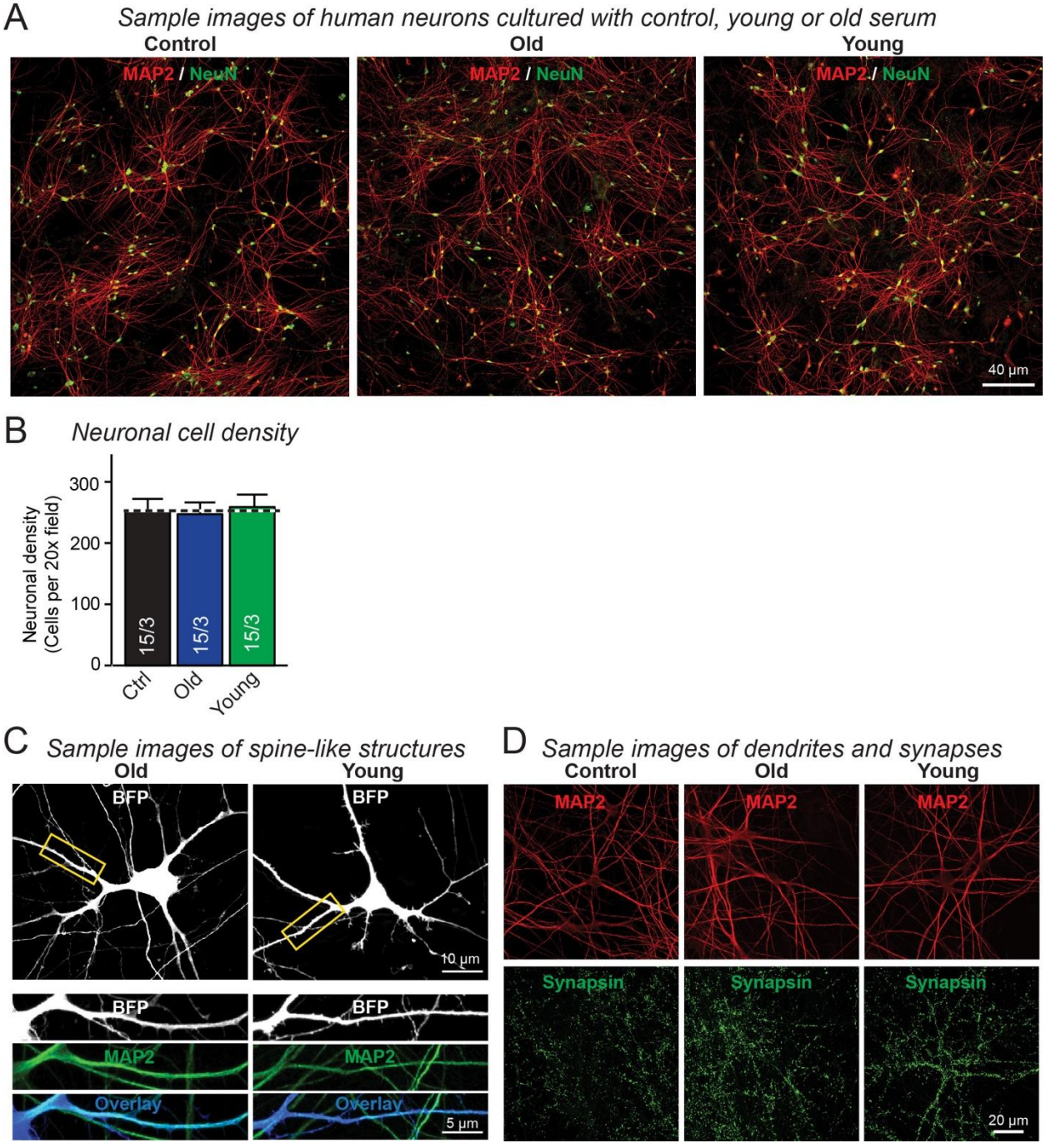


Figure S1: **Young and old serum do not significantly alter overall morphology or survival of human neurons compared to fetal bovine serum, but young serum dramatically increases formation of spine-like structures and synapses** (related to Figure 1-3)

(A) Representative low-magnification images of human neurons cultured in fetal bovine serum (FBS; 'control') or old or young serum from mice. Neurons were incubated with serum starting at

DIV10 and immunostained for MAP2 as a dendritic marker and NeuN as a neuronal marker at DIV35.

(B) Human neurons cultured in FBS or old or young serum exhibit no difference in cell density at DIV35 (data are means ± SEM; numbers of cells/ independent cultures analyzed are shown in bars). Statistical analyses performed by one-way ANOVA followed by Tukey's post-hoc comparisons revealed no significant differences.

(C) Young serum generates dendritic spine-like protrusions as illustrated by representative images. Neurons transfected with eBFP were treated with FBS (control) or young or old serum and counterstained for MAP2. Spine-like protrusions are filled with eBFP but are devoid of MAP2.

(D) Young serum increases synapse density as visualized by immunostaining for MAP2 and synapsin. Images show representative neurons treated with FBS (control) or young or old serum (for merged images and quantifications, see Figure 1G, 1H).

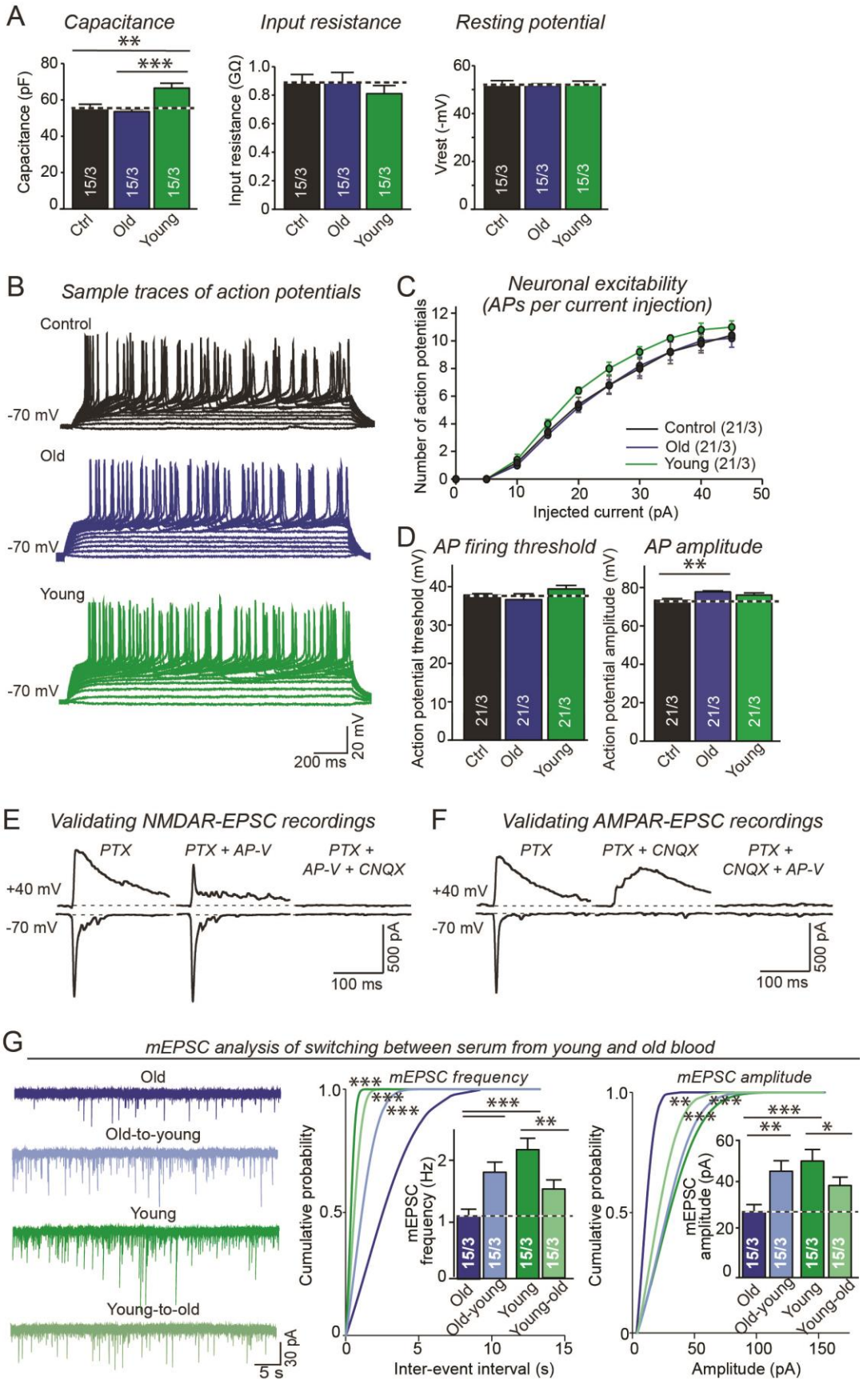

A
Capacitance
Input resistance
Resting potential
Capacitance (pF)
Input resistance (GΩ)
Vrest (-mV)
15/3
Ctrl
Old
Young
B
Sample traces of action potentials
Control
-70 mV
Old
Young
200 ms
20 mV
C
Neuronal excitability
(APs per current injection)
Number of action potentials
Injected current (pA)
Control (21/3)
Old (21/3)
Young (21/3)
D
AP firing threshold
AP amplitude
Action potential threshold (mV)
Action potential amplitude (mV)
21/3
E
Validating NMDAR-EPSC recordings
PTX
PTX + AP-V
PTX + AP-V + CNQX
+40 mV
-70 mV
500 pA
100 ms
F
Validating AMPAR-EPSC recordings
PTX
PTX + CNQX
PTX + CNQX + AP-V
G
mEPSC analysis of switching between serum from young and old blood
Old
Old-to-young
Young
Young-to-old
30 pA
5 s
mEPSC frequency
mEPSC amplitude
Cumulative probability
Inter-event interval (s)
Amplitude (pA)
mEPSC frequency (Hz)
mEPSC amplitude (pA)
Old
Old-young
Young
Young-old

Figure S2: **Young serum increases the membrane capacitance of human neurons without significantly altering their passive and active membrane properties (A-D), validation of selective AMPAR- and NMDAR-EPSC measurements by pharmacological isolation (E & F), and effects of young and old serum exchange on spontaneous synaptic activity** (related to Figure 1-3)

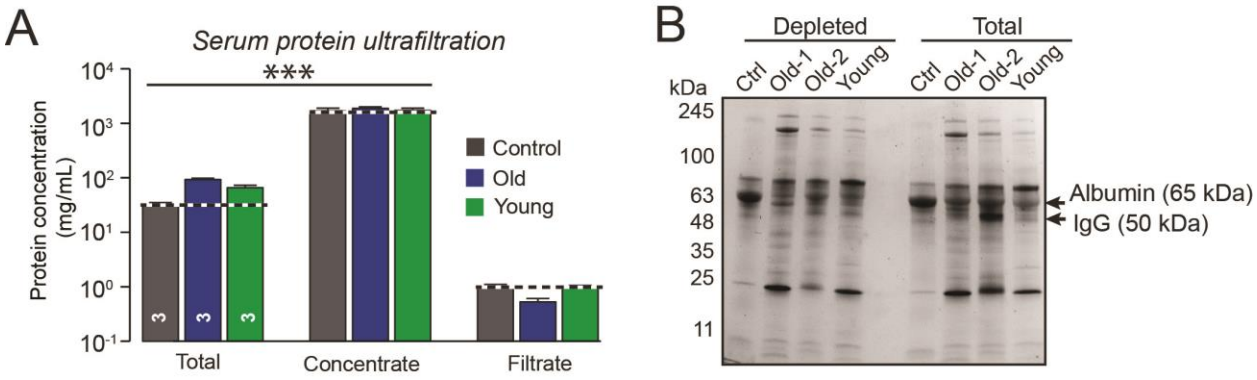
(A) Young serum significantly increases the membrane capacitance of human neurons, but neither young nor old serum produces significant differences in input resistance or resting membrane potential in human neurons.

(B-D) Human neurons cultured in FBS or old or young serum exhibit no significant differences in membrane excitability and action potential firing properties (D, representative traces of action potentials elicited by current injections of increasing strength (10-pA increments) in current-clamp mode; E, plot of action potentials as a function of injected currents; F, summary graphs of action potential firing thresholds and amplitudes).

(E & F) Representative traces of evoked EPSCs recorded as a function of inhibitors of AMPARs and NMDARs. Human neurons were cultured in young serum, and EPSCs were recorded at DIV42 in the presence of picrotoxin (PTX; 50 µM) at -70 mV and +40 mV holding potentials. The effect of the sequential addition of the NMDAR-blocker AP-V (50 µM) and of the AMPAR-blocker CNQX (20 µM) was monitored in E, whereas the effect of the reverse order of addition was monitored in F. AMPAR-EPSC amplitudes were quantified as peak amplitudes at a holding potential of -70 mV, while NMDAR-EPSC amplitudes were quantified as the amplitudes at 50 ms after the initial stimulus at a holding potential of +40 mV. The representative traces shown demonstrate that these measurements are selective for AMPAR- and NMDAR-EPSCs, respectively.

(G) Young serum increases spontaneous synaptic activity in neurons that were previously exposed to old serum as assessed by mEPSC recordings. Neurons were cultured in young or old serum from DIV10, switched at DIV35 to the other type of serum, or kept as a control in the original type of serum. Neurons were analyzed at DIV42 (left, representative traces; right: cumulative probability plots of mEPSC interevent intervals and of mEPSC amplitudes [insets: summary graphs of the mEPSC frequency and mEPSC amplitude, respectively]).

All bar graphs and summary plots represent means ± SEM; numbers of cells/ independent cultures analyzed are shown in bars. Statistical analyses were performed by one-way ANOVA followed by Tukey's post-hoc comparisons (** = $0.05<p<0.01$; *** = $p<0.001$). Non-significant comparisons are not shown.



C

Mass spectrometry of proteins in serum from young vs. old serum

	Protein	Gene	No. proteins		No. peptides		Ratio Sample:Pooled		Fold Elevation		Molecular Function	Subcellular Localization	Expression
Enriched in young serum	α2HS-glycoprotein	AHSG	3	2	15	14	1.9	2.1	1.9	3.5	Cysteine-type endopeptidase inhibitor	Secreted	Liver, limb buds
	Lumican	LUM	1	1	9	13	1.4	1.5	1.6	2.1	Collagen binding	Secreted	Cornea, umbilical cord, bones
	Thrombospondin-4	THBS4	1	1	8	10	1.7	1.6	2.5	2.6	Binding to calcium, heparin, integrin	Secreted	Skeletal muscle, brain, liver
	α-fetoprotein	AFP	4	4	5	12	2.4	2.4	9.3	7.0	Binding to estrogens, fatty acids, metal ions	Secreted	Plasma
	SPARC_like protein 1 (a.k.a. Hevin)	SPARCL1	2	2	4	5	1.5	1.1	1.7	2.3	Binding to calcium, collagen, ECM	Secreted	Brain, heart, adrenal gland, lung, kidney, liver
	Comp protein	COMP	3	3	3	3	0.8	0.9	2.2	1.7	Binding to calcium	Secreted	Unknown
Enriched in old serum	Pregnancy zone protein	PZP	1	1	91	111	1.4	1.6	2.1	1.9	Serine-type endopeptidase inhibitor	Secreted	Liver, ovary, heart, stomach
	Complement factor H	CFH	2	1	62	54	1.6	1.2	1.8	1.2	Unknown	Secreted	Unknown
	α1β glycoprotein	A1BG	1	1	23	23	2.9	2.7	15.4	18.6	Unknown	Secreted	Liver
	Complement factor I	CFI	1	1	23	16	1.3	0.9	2.7	1.8	Serine-type endopeptidase, metal ion binding, scavenger receptor	Secreted	Plasma

Figure S3: **Partial purification of proteins from young and old serum using ultracentrifugation and subsequent depletion of albumin and immunoglobulin, and tandem mass tag labeling and mass spectrometry of fractionated serum proteins** (related to Figure 4-7)

(A) Isolation of proteins from FBS (control) and from old and young mouse sera by centrifugal ultrafiltration through an Amicon cellulose membrane with a 3-kDa molecular weight cut-off. Bar graphs depict mean protein concentrations in total sera, concentrate and filtrate as measured by a colorimetric BCA protein assay (means ± SEM; n = 3 independent serum collections). Statistical significance (***p<0.001) was evaluated by two-way ANOVA and Tukey's post-hoc test. Non-significant comparisons are not indicated.

(B) Representative SDS-gel of total sera (Total) and of the serum fractions obtained after ultracentrifugation of serum samples through an Amicon filter and subsequent depletion of albumin and immunoglobulin (IgG) from the filtration concentrate by chromatography on an albumin- and immunoglobulin-binding column (Depleted). Proteins were analyzed by SDS-PAGE on a TGX Stain-Free gel and visualized by UV illumination.

(C) Table of proteins enriched in young or old serum as identified by tandem mass tag (TMT) labeling and mass spectrometry of fractionated serum proteins. Candidates were selected in an unbiased manner by ranking protein groups according to coverage and calculating reciprocal fold-elevation ratios from protein abundances in each sample to reveal their enrichment in old or young blood (cut-off ratio = 1.5). Results from two independent mass spectrometry experiments are shown.

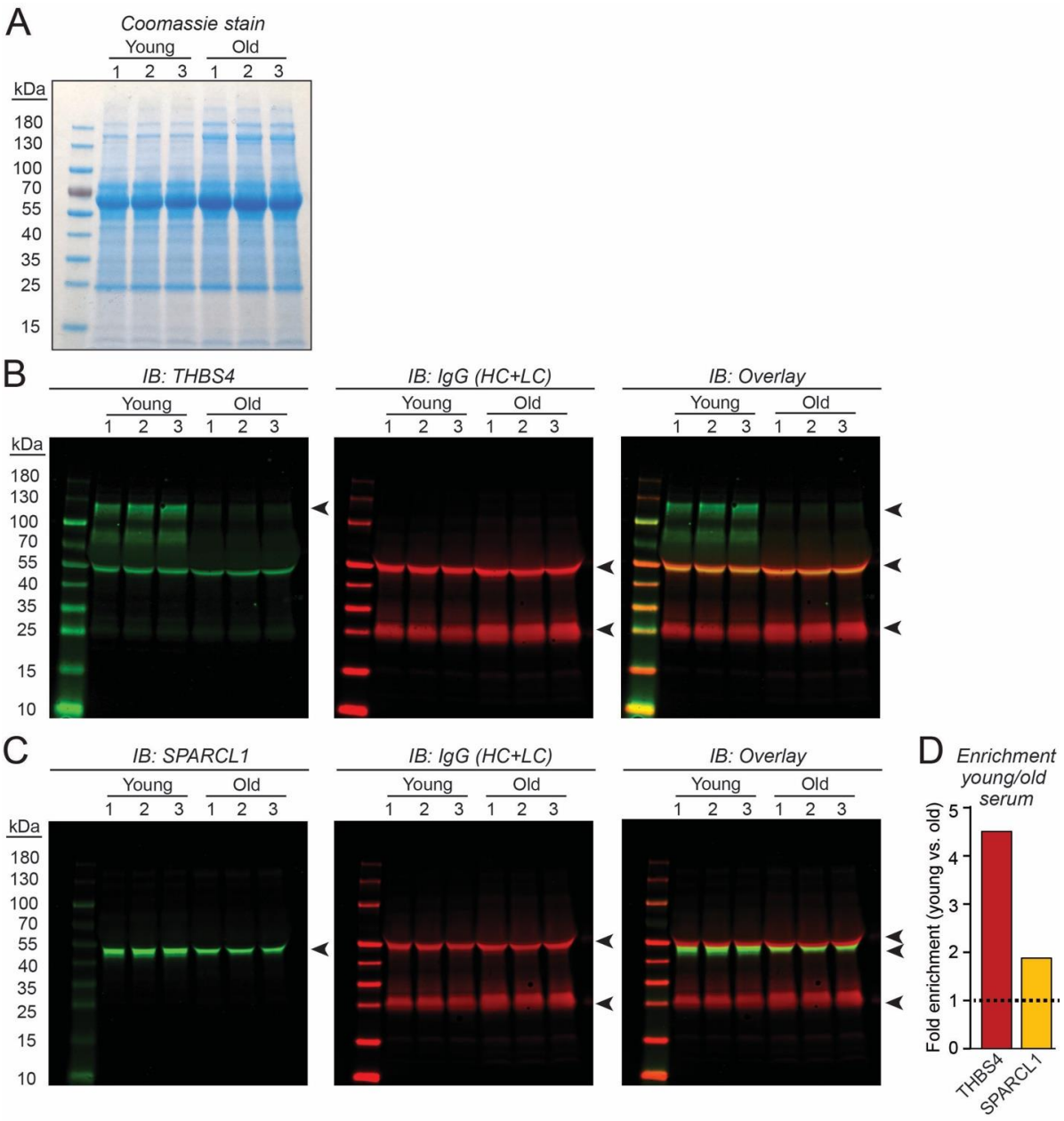


Figure S4: **Measurements of the relative concentrations of THBS4 and SPARCL1 in young and old mouse serum** (related to Figure 4)

(A) Coomassie-stained gel of serum isolated from young (15-day old) and old (12-month old) mice. Equal volumes of three independent samples were analyzed.

(**B** & **C**) Representative immunoblots of the three independent serum samples from young and old mice examined as in panel A, but probed with antibodies to THBS4 and IgG (B) or to SPARCL1 and IgG (C). The IgG signal was used as an internal standard to control for the higher concentration of serum proteins in old mice.

(D) Enrichment of THBS4 and SPARCL1 in young versus old serum, quantified based on the data shown in Figure 4E.

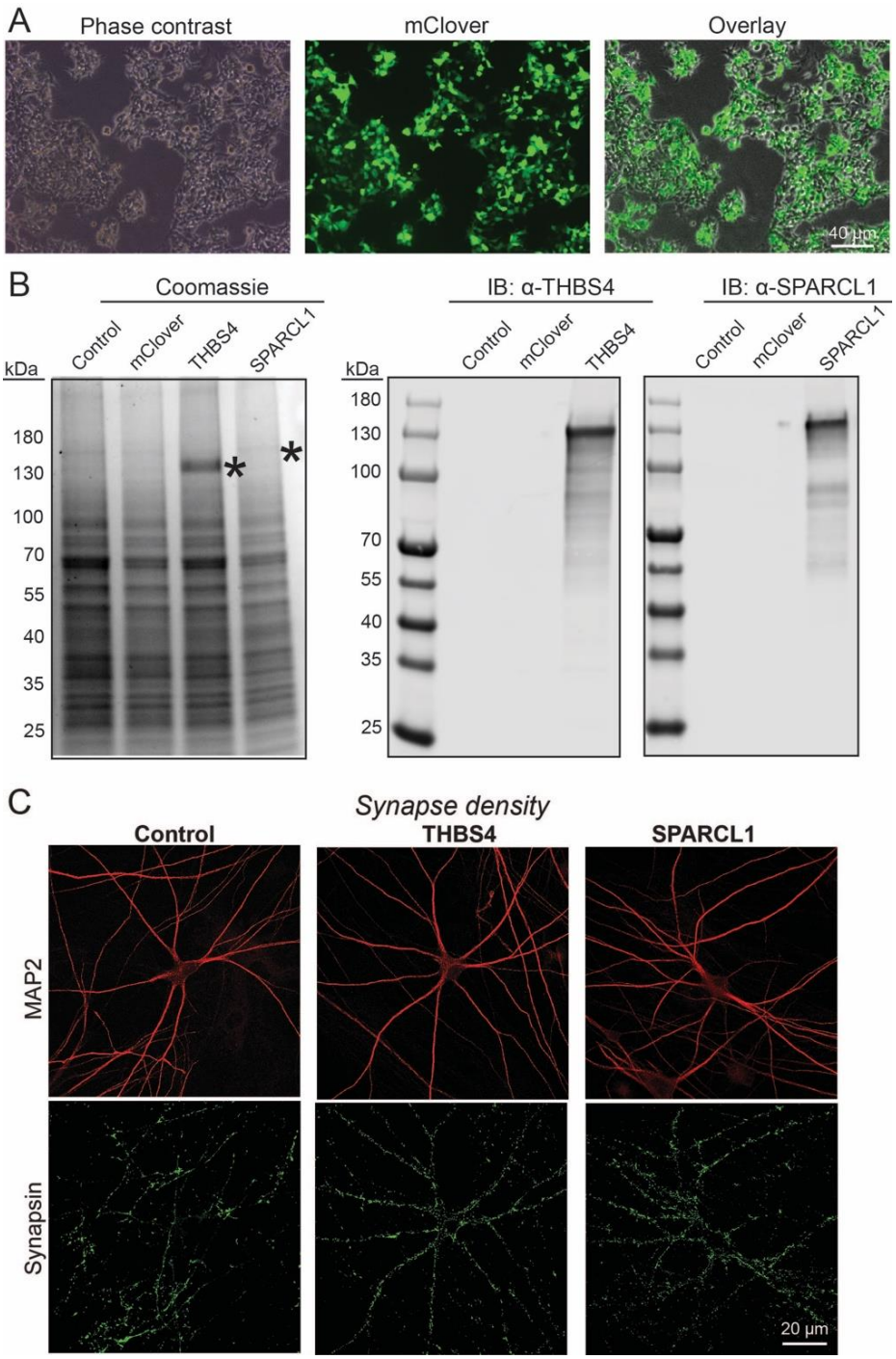


Figure S5: **Production of recombinant thrombospondin-4 (THBS4) and SPARCL1 in transfected HEK293T cells** (related to Figure 5-8)

(A) Representative images of HEK293T cells expressing mClover, serving as a positive control for transfection of plasmids encoding the recombinant factors.

(**B**) Production of THBS4 in transfected HEK293 cells as analyzed by SDS-PAGE of the proteins secreted into the medium and stained with Coomassie blue (left) or detected by immunoblotting

for THBS4 or SPARCL1 (right). Note that THBS4 is produced in copious amounts, whereas SPARCL1 is only detectable by immunoblotting (asterisks in image of the Coomassie-stained gel identify THBS4 and SPARCL1 bands).

(C) THBS4 and SPARCL1 increase synapse density in neurons treated with recombinant proteins as described for A & B (C, representative images of control and treated neurons immunostained for MAP2 and synapsin).

Supplementary Information References

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3. Patzke C, Acuna C, Giam LR, Wernig M, Südhof TC (2016) Conditional deletion of L1CAM in human neurons impairs both axonal and dendritic arborization and action potential generation. J Exp Med 213, 499-515.
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